SOLUBILIZATION OF AUSTRALIAN LIGNITES BY MICROORGANISMS

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INTRODUCTION

Cohen and Gabriel (1982) found that the wood rot fungi *Polyporus versicolor (Trametes versicolor)* and *Poria monticola (Poria placenta)* were able to solubilize Leonardite, a naturally oxidized lignite from a deposit in North Dakota. Lignite pieces placed on the surface of mycelial mats of each species, growing on Sabourauq maltose agar, were reduced to black water soluble products, shown by Wilson *et al* (1987) to contain aromatic, carboxylic and aliphatic carbon and have molecular weights of 10,000 to 50,000 Daltons or more.

Other North American lignites were found to be much less susceptible to solubilization by both these and other fungi (Ward 1985_1 and Scott *et al* 1986). However, Strandberg and Lewis (1986) showed that a range of lignites and a subbituminous coal could be solubilized by Candida (ML13) following oxidation with acid, peroxide or ozone. Several lignin degrading organisms have also been shown to solubilize oxidized lignite, including: *Phanerochaete chrysosporium* (Scott and Lewis 1987), *Streptomyces setonii* and *S. viridosporus* (Strandberg and Lewis 1987). Ward (1985_2) was able to isolate a range of lignite degrading fungi from a naturally exposed lignite seam. These included species of Aspergillus, Candida, Mucor, Paecillomyces and Penicillium. The mechanisms involved are not clear and may not all reflect enzymatic breakdown of lignin like structures remaining in lignite. Fakoussa and Truper (1983) have reported that surfactants produced by the bacterium *Pseudomonao fluorescens* have a solubilizing action on coal.

Australia has substantial lignite deposits, particularly in the Latrobe Valley in Victoria where 4.10^{10} tonnes are accessible with available technologies (Perry *et al* 1984). We have investigated the susceptibility of these coals to solubilization by microorganisms, including species additional to those already identified as active on North American lignites.

MATERIALS AND METHODS

Cultures were obtained from: The American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, USA (ATCC 11538, 12679 and 24725); CSIRO Division of Chemical and Wood

Technology, P.O. Box 56, Highott, Victoria 3190, Australia (DFP 7522); Dr. G.W. Strandberg, Chemical Technology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA. *(Streptomyces viridosporus)*; and Dr. B. Ward, Department of Biology, University of Mississippi, University, MS 38667, USA (ML-13, ML-20 and YML-1).

Solubilization tests were conducted at 28°, at a relative humidity exceeding 80%, in 250ml screw capped jars containing 50ml of Sabouraud maltose agar. Tho jar closures had a 10mm perforation plugged with non-absorbent cotton. For *Trametes versicolor*, lignite pieces were placed on the surface of the mycelial mat, 8 days after central inoculation of the medium. For other tests, coal pieces were added at the same time as a spread inoculum of a suspension of conidia or mycelial fragments. Lignite solubilization was detected by the appearance of pigmented droplets on the surface of the coal pieces and by diffusion of dark solubilization products into the medium. The degree of solubilization was quantitated by recovery of remaining lignite pieces which were washed in deionized water, dried at 95° and weighed.

Lignite samples were obtained from the Coal Corporation of Victoria. Run of mine coal came from a 20,0001 sample taken at Morwell in October 1982 and a 40,0001 sample taken at Loy Yang in October 1984. Both had been stored in sealed drums. Weathered lignite came from a deposit at Loy Yang. The samples were pushed through a 4mm screen and fines passing a 2.4mm mesh removed. Lignite was sterilized by autoclaving at 121° and dried to constant weight at 95° prior to use. Lignite was oxidized by reacting with 8M nitric acid for 18 hours, washed with water and dried. Approximately 300mg dry weight of lignite was used for each test.

RESULTS

Mycelial mats of *Trametes versicolor* were able to solubilize both Latrobe Valley lignites following acid oxidation (Table-1). Run of mine lignite was resistant, with the exception that Loy Yang coal showed trace coloration of droplets forming on the coal pieces and diffusion of dark material into the agar surrounding coal fragments. Pigmented droplets formed within 14 days both on acid oxidized Morwell and acid oxidized Loy Yang lignites. There was extensive collapse of the granules by day 38. The incubation was continued to day 118 prior to quantitation. Although pigmented droplets appeared on the Loy Yang run of mine coal at 42 days, there was no extensive solubilization. Mass gain by unoxidized lignite pieces reflects the difficulty of separating them from the pigmented droplets formed and no pigments diffused into the agar in cultures without lignite or when lignite was in contact with medium under aseptic conditions.

Seven strains of six additional species were tested for their effect on acid oxidized and run of mine Morwell lignite, and on a naturally oxidized, weathered, lignite deposit at Loy Yang, Table-2. Each of these species, previously shown to be active on oxidized North American lignites, were able to solubilize acid oxidized Morwell lignite. *Poria placenta* was least effective and the ATCC 11538 strain reported active on Leonardite (Cohen and Gabriel 1982) did not solubilize any of the Latrobe Valley lignites in repeated tests. A strain of *P. placenta* from the CSIRO collection showed some activity on acid oxidized Morwell coal by the diffusion test. Each of the other five species caused extensive or total solubilization of acid oxidized lignite and were also active on the naturally oxidized weathered coal on which *Phanerochaete chrysosporium* was the most effective. None of the isolates tested showed evidence of activity on run of mine coal, with the exception of Candida ML-13 that was positive by the diffusion test but so heavily interpenetrated the coal grains that quantitation of the extent of solubilization was not possible.

DISCUSSION

The data presented here show that acid oxidized lignites from the Latrobe Valley are solubilized by each of seven species of microorganisms previously found to be active on Leonardite and oxidized North American lignites. These are the wood rot fungi: *Trametes versicolor, Poria placenta and Phanerochaete chrysosporium,* the lignin degrading prokaryote *Streptomyces viridosporus* and three fungi isolated from lignite in Mississippi: Candida ML-13, Cunninghamelia YML-1 and *Penicillium waksmanii*.

The completeness of solubilization by these organisms was not correlated with the time of onset of solubilization as indicated by the diffusion of pigment from lignite grains, data not shown, and varied from as soon as 5 days after inoculation in Cunninghamella, that gave 74% solubilization by day 67, to as late as 46 days after inoculation by *Penicillium waksmanii* which gave 100% solubilization by day 67. This suggests that there is substantial potential for the manipulation of conditions and of the organisms themselves to speed solubilization. It should be noted that the extended incubation periods used in these tests do not reflect the speed of the process by the most active species and were chosen such that weak or delayed activity could be detected. It is desirable that even slow acting isolates are identified since they may include species degrading lignite by different mechanisms. Identification of all biological mechanisms of lignite degradation may provide pathways to desirable products that can be manipulated to give good yields at practicable rates.

Further to the work reported here, we have surveyed other lignin and wood degrading species for their activity on Latrobe Valley lignites. Of the lignin degraders, four were inactive but the remaining six solubilized acid oxidized Morwell lignite and also weathered lignite to a substantial extent. Three of these species also showed some activity on run of mine Morwell lignite. In addition, we have established enrichment cultures for lignite degrading species from natural lignite exposures and from mining sites in Victoria. These enrichments have yielded a wide range fungi, Streptomycetes and bacteria able to grow on lignite as their sole carbon source. The isolates include some able to quantitatively solubilize acid oxidized Morwell lignite and some that partially solubilize unoxidized, run of mine Morwell coal. This work has identified a second deposit of lignite at Loy Yang, that like Leonardite is readily solubilized by microorganisms without further treatment.

ACKNOWLEDGEMENTS

This work was supported by grants from the Coal Corporation of Victoria and from The Flinders University of South Australia. We thank Diana Thompson and Karen Ribbons for their skill and patience in separating lignite pieces from microbial growth.

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TABLE-1 ACTION OF *TRAMETES VERSICOLOR* ON RUN OF MINE AND ACID OXIDIZED AUSTRALIAN LIGNITES.

LIGNITE		COLOURATION OF DROPLETS ON COAL	COLOURATION OF AGAR	MASS CHANGE OF COAL %
MORWELL	ROM	NONE	NONE	+5
	OXIDIZED	BLACK	BLACK	-90
LOY YANG	ROM	TRACE	TRACE	+4
	OXIDIZED	BLACK	BLACK	-83

TABLE-2 SOLUBILIZATION OF MORWELL AND LOY YANG LIGNITE BY MICROORGANISMS.

		LIGNITE					
		MORWELL ROM		MORWELL OXIDIZED		LOY YANG WEATHERED	
		a	*b*	*a*	*b*	*a*	*b*
Candida sp.	ML-13	B	+14	B	-74	B	-13
Cunninghamella sp.	YML-1	0	+1	B	-74	B	-1
Phanerochaete chrysosporium	ATCC24725	0	+1	B	-35	B	-47
Poria placenta	ATCC11538	0		0		0	
	DFP7522	0		B		0	
Penicillium waksmanii	ML-20	0	+2	B	-100	B	-3
Streptomyces viridosporus	T7A	0	+9	B	-75	B	-10

a Formation of pigmented droplets on coal pieces and/or diffusion of black pigment from the coal into the agar: 0 not observed, B black pigment.

b Percentage mass change of lignite pieces following 58 to 70 days incubation. Apparent mass gain is due to mycelium penetrating the lignite pieces.